

Method based on S. T. K. my et al., Bioc. Biotech. Biodynam. 1096-  
1099 (1995) 822-826.

Reagents: Tris-maleic acid buffer 55. with Tris-25.6 g.a.,  
pH 8.2 - RT. made. = TCB solution

40mM Pyrogallol in 10mM HCl. 10mM dH<sub>2</sub>O + 8.6mL  
11.6N HCl + 50.5mg pyrogallol (m.w.170)

\* Tris 2mM pyr. first  $\rightarrow$  no absorbance A.

Reaction: 1. 8mL TCB solution

• 2mL Pyrogallol solution

• 1mL SOD (10<sup>6</sup>/mL) or 0.1mL H<sub>2</sub>O

2. 1mL

Vortex, pipet rapidly into cuvette.

Kinetic protocol  $\rightarrow$  420nm, RT, blanked again  
H<sub>2</sub>O. SOD/TCB blank = H<sub>2</sub>O absorbance  
at 420nm.

Plan: 2mL reaction + C<sub>3</sub> dose-response:

0, 10, 10<sup>1</sup>, 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup>, 10<sup>6</sup>, 10<sup>7</sup>, 10<sup>8</sup>

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Add as 0.1mL solutions.

C<sub>3</sub> 1 = 0.1mL TCB

10<sup>1</sup> =  $x(2.5mM)(2.1mL)(10\muM)$   $x = 0.84\muL + 99\muL$  TCB

30<sup>1</sup>

$x = 2.5\muL + 97\muL$  TCB

100<sup>1</sup>

$x = 8.4\muL + 92\muL$  TCB

300<sup>1</sup>

$x = 25\muL + 7.5\muL$  TCB

500<sup>1</sup>

$x = 42\muL + 5.8\muL$  TCB

500<sup>1</sup>  $\rightarrow$  absorbance off-scale

Some  $\downarrow$  SOD. Try 20mM Pyr. and 10mM Pyr.  $\infty$   
same C<sub>3</sub> conc.

10mM pyr.  $\rightarrow$  2mL 40mM Pyr. + 6mL 10mM HCl

\* Need to run + SOD control against 10mM Pyr.

EXHIBIT